Remarks

Claims 1-28 were pending in the subject application. By this Amendment, claims 1-28 have been canceled, and new claims 29-41 have been added. The undersigned avers that no new matter is introduced by this amendment. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 29-41 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

By this Amendment, the applicants have cancelled claims 1-28 and added claims 29-41. Support for claim 29 can be found, for example, at page 4, paragraph 11; pages 29-30, paragraph 64; page 31, paragraph 65; and claim 24 as originally filed. Support for claim 30 can be found, for example, at page 4, paragraph 11, and claim 25 as originally filed. Support for claim 31 can be found, for example, at page 4, paragraph 10; page 34, paragraph 71; Figure 8; and claim 18 as originally filed. Support for claim 32 can be found, for example, at page 34, paragraph 71; Figure 8; and claim 18 as originally filed. Support for claims 33-38 can be found, for example, at pages 5-6, paragraphs 21-23; pages 29-30, paragraph 64; and page 31, paragraph 65. Support for claims 39-40 can be found, for example, at page 1, paragraph 3; page 4, paragraph 9; pages 22-23, paragraphs 55-56; page 34, paragraph 71; Figure 8; page 38, paragraph 76; and claims 19 and 26 as originally filed. Support for claim 41 can be found, for example, in claim 27 as originally filed. The applicants submit that claims 29-41 are consonant with the election of Group V set forth in the restriction requirement (claims 18, 19, and 24-27, drawn to a method for inducing proliferation by introducing an inhibitor of s-SHIP activity and wherein the proliferating stem cells are induced to differentiate).

Claims 18, 19, and 24-27 are rejected under 35 U.S.C. §112, first paragraph, as non-enabled. The applicant respectfully traverses and submits that the claims are fully enabled by the subject specification.

As taught in the specification, there is discordance between stem cells and mature hematopoietic cells in the expression of s-SHIP mRNA. Membrane localization of s-SHIP and its association with a major adaptor protein indicate a role for s-SHIP in signaling pathways active in stem cells. The subject invention involves reducing s-SHIP function in stem cells to induce cellular proliferation. As indicated above, claims 18, 19, and 24-27 have been cancelled and new claims 29-

41 have been added. Claim 29 recites that the inhibitor induces proliferation of human or mouse stem cells. Furthermore, dependent claim 31 recites that the inhibitor is an anti-s-SHIP shRNA that reduces s-SHIP expression in the stem cells.

The Office Action indicates that the subject specification does not provide sufficient guidance to teach one skilled in the art how to make shRNA that targets SHIP RNA and use the shRNA to induce cellular proliferation. At page 3, the Office Action sites various issues concerning mRNA site selection, delivery, and intracellular localization. As acknowledged by the Examiner, various gene delivery vehicles have been used to deliver nucleic acids. For example, viral and non-viral vectors, such as polycationic molecules (*e.g.*, liposomes), can be used to deliver genetic constructs. DOTAP has been used for gene delivery to mammalian cells *in vitro* and *in vivo* (see, for example, Porteous D.J. *et al.*, "Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis", *Gene Ther.*, 1997, Mar., 4(3):210-218; Song Y.K. *et al.*, "Characterization of cationic liposome-mediated gene transfer *in vivo* by intravenous administration", *Hum. Gene Ther.*, 1997, Sept., 8(13):1585-1594).

RNAi has been demonstrated to facilitate gene silencing in a variety of cell types (primary cells and cell lines) and animal models (see, for example, Tuschl *et al.*, *Mol. Interv.*, 2002, 2(3):158-167, particularly Table 1; Oliveira *et al.*, *Genesis*, 2003, 36(4):203-208 (abstract only); Reich *et al.*, *Molecular Vision*, 2003, May, 9:210-216; Barton *et al.*, *PNAS*, 2002, 99(23):14943-14945; Peng *et al.*, *Cancer Research*, 2002, 62:6400-6404; McManus *et al.*, *J. Immunology*, 2002, 169:5754-5760; Yang *et al.*, 2002, *PNAS*, 99(15):9942-9947; Donze *et al.*, 2002, *Nucleic Acids Research*, 30(10)e46:1-4; Krichevsky *et al.*, *PNAS*, 2002, 99(18):11926-11929, which are submitted herewith for the Examiner's consideration). Also submitted herewith for the Examiner's consideration are Milhavet *et al.* ("RNA Interference in Biology and Medicine", *Pharmacol. Rev.*, 2003, Dec., 55(4):629-648) and Agrawal *et al.* ("RNA Interference: Biology, Mechanism, and Applications", *Microbiol. Mol., Biol. Rev.*, 2003, Dec., 67(4):657-685). Although the Milhavet *et al.* and Agrawal *et al.* publications appear to have been published shortly after the subject application's filing date, these publications are review articles citing several earlier papers relating to the design, synthesis, and delivery of interfering RNA molecules. Thus, the Milhavet *et al.* and Agrawal *et al.* publications are submitted herewith to demonstrate the state of the art at the time the application was filed. See,

for example, pages 634 to 640 of Milhavet *et al.* and pages 671 to 672 of Agrawal *et al.* As is made clear from the Milhavet *et al.* and Agrawal *et al.* publications, many laboratories have had great success in reducing endogenous gene expression in a large variety of cell types, using various interfering RNA species and delivery methods (see, for example, Table 1 at pages 635-636 of Milhavet *et al.*).

Background information regarding various SHIP isoforms is provided in Rohrschneider *et al.* (*Genes & Development*, 2000, 14:505-520), which is submitted herewith for the Examiner's consideration. The mRNA sequences of mouse s-SHIP and the human ortholog of s-SHIP (SIP-110) are disclosed in the subject specification. Moreover, the mouse and human s-SHIP sequences have been publicly available since the late 1990s, as evidenced by accession numbers AF184912 and U50040, from the National Center for Biotechnology Information (NCBI) database, which are submitted herewith. The structure of the mouse and human s-SHIP genes is also described in the specification (see, for example, pages 31-32, 35-36, and Figures 1C, 2, 3A and 3B, and 7). Having the structure and sequence of the target gene (s-SHIP), the applicants submit that one skilled in the art can readily identify target nucleic acid sequences within the recipient mouse or human mRNA.

Furthermore, due to the certainty of the genetic code and complementarity, there is a well known correlation between target nucleic acid sequences within a target gene and nucleic acid sequences that interfere with the expression of the target gene. Hence, having the nucleotide sequence of the target gene provides sufficient information to one skilled in the art to obtain interfering RNA molecules. Due to nucleotide complementarity and the mechanism of RNA interference (RNAi), RNA molecules likely to hybridize with s-SHIP mRNA and interfere with its expression could then be determined. One of ordinary skill in the art need only be provided with the sequence of the target gene, as opposed to the sequence of any particular interfering RNA. There is no information essential for carrying out the invention that is not provided in the specification or not well known to those skilled in the art. As indicated by Milhavet *et al.*,

> All that is needed to implement siRNA-mediated silencing of expression of a gene of interest is the cDNA sequence of that gene, and commercially available reagents with which to perform the synthesis (Milavet *et al.* page 637, column 1, lines 2-6).

While it is true that not all shRNA molecules will inhibit a target gene, the availability of target gene sequence information, the capability to synthesize potentially interfering RNA molecules

in large quantities, and the availability of parameters and guidelines for selection of mRNA target sequences increase the likelihood of obtaining gene silencing RNA molecules. Thus, while the predictability that any single interfering RNA molecule will be effective is not necessarily high, the probability of finding an individual functional interfering RNA molecule is high. Summaries of these criteria for selection of interfering RNA and target mRNA sequences are provided in the Agrawal *et al.* (page 671, paragraph bridging the first and second columns) and Milhavet *et al.* (page 637, first column, lines 6-29) publications. Furthermore, computer algorithms are available to further optimize selection of interfering RNA sequences (Agrawal *et al.*, page 671, second column, lines 3-8). In addition, various expression and parallel analysis techniques are available to facilitate large-scale, high-throughput screening (see, for example, Sohail *et al.*, *Nucleic Acids Research*, 2003, April, 31(7):e38; Castanotto *et al.*, *RNA*, 2002, 8:1454-1460; and Mousses *et al.*, *Genome Res.*, 2003, October, 13(10):2341-2347, which are submitted herewith for the Examiner's consideration).

All the information necessary for one of ordinary skill in the art to carry out the method of the invention is disclosed in the application. It has been held that even if the practice of a method requires a particular apparatus, the application must provide a sufficient disclosure of the apparatus if the apparatus is not readily available. *In re Ghiron*, 442 F.2d 985, 991; 169 USPQ 723, 727 (CCPA 1971). The same can be said if certain chemicals are required to make a compound or practice a chemical process. *In re Howarth*, 654 F.2d 103, 105, 210 USPQ 689, 691 (CCPA 1981) and MPEP 2164.01(b). As shown by the publications submitted herewith, materials and methods for selection, synthesis, and delivery of interfering RNA molecules were commercially available at the time the application was filed.

> It is well settled that the disclosure of an application embraces not only what is expressly set forth in words or drawings, but what would be understood by persons skilled in the art. As was said in *Webster Loom Co. v. Higgins et al.*, ...the applicant 'may begin at the point where his invention begins, and describe what he has made that is new and what it replaces of the old. That which is common and well known is as if it were written out in the patent and delineated in the drawings'. *In re Howarth*, 210 USPQ 689, 692 (C.C.P.A. 1981) (emphasis added).

Not everything necessary to practice the invention need be explicitly disclosed in the application. *In re Buchner*, 929 F.2d 660, 661; 18 USPQ2d 1331, 1332 (Fed. Cir. 1991) and MPEP

2164.08. All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art. As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied. *In re Fisher*, 427 F.2d 833, 839; 166 USPQ 18, 24 (CCPA 1970). Failure to disclose other methods by which the claimed invention may be made does not render a claim invalid under 35 U.S.C. §112. *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1533; 3 USPQ2d 1737, 1743 (Fed. Cir.), cert. denied, 484 U.S. 954 (1987) and MPEP 2164.01(b). The specification need not even contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908; 164 USPQ 642, 645 (CCPA 1970) and MPEP 2164.02.

At pages 4-7 of the Office Action, the Examiner raises issues related to the phenotype exhibited by cells with reduced SHIP function. The Okada *et al.* publication pertains to SHIP-deficient DT40 cells. Not only are these cells differentiated B cells, as acknowledged by the Examiner, they are avian (chicken) cells. Therefore, the applicants submit that the cells in the Okada *et al.* publication convey no information concerning s-SHIP deficiency in stem cells. Furthermore, claim 29 recites a method for inducing proliferation of human or mouse stem cells, comprising introducing an inhibitor of murine s-SHIP activity or human s-SHIP (SIP-110) activity. Thus, the characteristics of SHIP-deficient DT40 cells are of little relevance to the method of the invention. The Liu *et al.* publication describes the use of two SHIP constructs lacking a functional SH2 domain which did not appear to affect cell proliferation. At page 5, the Office Action indicates that "functionally, it would seem that these constructs are similar to s-SHIP" and, based on the results of Liu *et al.*, "there should be no reason to imply that the use of shRNA to target any SHIP affects cell proliferation." Claims 29-41 do not recite the inhibition of "any SHIP". As indicated above, claim 29 recites a method for inducing proliferation of human or mouse stem cells, comprising introducing an inhibitor of murine s-SHIP activity or human s-SHIP (SIP-110) activity. The applicants respectfully submit that one of ordinary skill in the art would not correlate the effects of the SHIP constructs in Liu *et al.* with the role of s-SHIP merely based on the common lack of a functional SH2 domain. The constructs in Liu *et al.* and s-SHIP are not analogous; hence, this is an invalid

extrapolation. In addition, the Liu *et al.* publication used DA-ER cells, which are mouse myeloid leukemic cells engineered to overexpress erythropoietin (EPO) receptors, not stem cells (see the abstract and page 2297, first column, of Damen *et al.* (*Blood*, 1993, 82(8):2296-2303), which is submitted herewith). Therefore, it is also the wrong cellular context for such a comparison. In regard to the Helgason *et al.* publication, as indicated at paragraph 72 of the specification, the mutation strategy employed targets the full length form of SHIP, not the s-SHIP isoform. Although s-SHIP protein could not be detected from total bone marrow of SHIP-null mice in which the first exon of SHIP was insertionally mutated, this is an issue with the limit of (protein) detection associated with Western blot. The s-SHIP isoform is indeed present in these SHIP-null mice. Submitted herewith for the Examiner's consideration is Exhibit A, which shows results of RT-PCR carried out on whole bone marrow (BM) cells for detection of s-SHIP. s-SHIP expression was detected in the SHIP-/- BM cells at levels comparable to that in the wild-type C57BL/6 mice and BALB/C mice.

Claim 41 recites that the stem cells are induced to differentiate. The scientific literature is replete with reports on differentiating stem cells into various mature cell types *in vitro* and *in vivo*. Various culture media, culture techniques, and differentiation-inducing agents (such as hormones, interleukins and interferons, vitamins, and ions) are well known in the art and, thus, need not be listed in the subject specification. TGF-beta family members have remarkable instructive effects in both ES cell and neural crest stem cell differentiation (Shah *et al.*, *Cell*, 1996, 85:331; White *et al.*, *Neuron*, 2001, 29:57). In addition to secreted factors, integral membrane proteins as well as integrins and extracellular matrix (ECM) are also known to contribute to the microenvironment of stem cells in determining their fate (Watt *et al.*, *Science*, 2000, 287:1427). A report demonstrated the use of FGF-2, absorbic acid, sonic hedgehog (SHH) and FGF-8 to differentiate mouse ES cells *in vitro*, obtaining dopaminergic (DA) and serotonergic neurons in high yield (Lee *et al.*, *Nat. Biotechnol.*, 2000, 18:675). The generation of insulin-expressing cells from mouse ES cells, both *in vitro* and *in vivo*, has also been reported (Lumelsky *et al.*, *Science*, 2001, 292:1389).

Given the state of the art as demonstrated by the scientific publications submitted herewith, and the information provided in the subject specification and the experimental results obtained therewith, one of ordinary skill in the art can target and reduce expression of mouse and human s-

SHIP, without resort to undue experimentation. Thus, the applicants respectfully submit that the subject specification enables the methods currently claimed.

Accordingly, the applicants respectfully submit that, given the teaching of the specification and the state of the art in gene suppression using interfering RNA, one of ordinary skill in the art could carry out the claimed methods without the need for undue experimentation. In view of the foregoing remarks, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 18, 19, and 24-27 are rejected under 35 U.S.C. §112, first paragraph, as lacking sufficient written description. The applicants traverse and respectfully submit that the subject specification provides a sufficient written description of the claimed invention. However, as indicated above, claims 18, 19, and 24-27 have been cancelled and new claims 29-41 have been added.

The applicants respectfully submit that the subject specification provides a sufficient written description of the claimed subject matter. The Office Action indicates that the subject application does not indicate the distinguishing attributes identifying members of the genus comprising shRNA molecules that inhibit SHIP expression. Claim 29 recites that the inhibitor induces proliferation of human or mouse stem cells, and dependent claim 31 recites that the inhibitor is an anti-s-SHIP shRNA that reduces s-SHIP expression in the stem cells. The subject invention involves reducing s-SHIP function in stem cells to induce cellular proliferation. As indicated above, the mRNA sequences of mouse s-SHIP and the human ortholog of s-SHIP (SIP-110) are disclosed in the subject specification and have been publicly available since the late 1990s, as evidenced by accession numbers AF184912 and U50040. The structure of the mouse and human s-SHIP genes is also described in the specification (see, for example, pages 31-32, 35-36, and Figures 1C, 2, 3A and 3B, and 7). As indicated at page 30, paragraph 65 of the specification, sequence analysis using the Clustal W algorithm showed that human and mouse s-SHIP genes share 78% nucleotide identity and have an open reading frame (ORF) with 88% amino acid identity.

Having the sequence of the target gene (s-SHIP), one skilled in the art would readily envision target nucleic acid sequences with the recipient mouse or human mRNA. Therefore, the applicants respectfully submit that the subject specification provides sufficient information regarding the genus

of s-SHIP mRNA and the shRNA specific thereto. As the Examiner is aware, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. *In re Buchner*, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 221 USPQ 481, 489 (Fed. Cir. 1984).

As indicated above, due to the certainty of the genetic code and complementarity, there is a well known correlation between target nucleic acid sequences within a target gene and nucleic acid sequences that interfere with the expression of the target gene. Hence, having the nucleotide sequence of the target gene provides discerning information regarding the sequences of suitable interfering RNA molecules, and leads one of ordinary skill in the art to their selection. Due to nucleotide complementarity and the mechanism of RNAi, RNA molecules likely to hybridize with s-SHIP mRNA and interfere with its expression could then be determined. One of ordinary skill in the art need only be provided with the sequence of the target gene, as opposed to the sequence of any particular interfering RNA. There is no sequence information essential for carrying out the invention that is not provided in the specification or not well known to those skilled in the art. Again, as indicated by Milhavet *et al.*,

> All that is needed to implement siRNA-mediated silencing of expression of a gene of interest is the cDNA sequence of that gene, and commercially available reagents with which to perform the synthesis (Milavet *et al.* page 637, column 1, lines 2-6).

While it is true that not all shRNA molecules will inhibit a target gene, the availability of target gene sequence information, the capability to synthesize potentially interfering RNA molecules in large quantities, and the availability of parameters and guidelines for selection of mRNA target sequences increase the likelihood of obtaining gene silencing RNA molecules. Thus, while the predictability that any single interfering RNA molecule will be effective is not necessarily high, the probability of finding an individual functional interfering RNA molecule is high. Summaries of these criteria for selection of interfering RNA and their mRNA targets are provided in the Agrawal *et al.* (page 671, paragraph bridging the first and second columns) and Milhavet *et al.* (page 637, first column, lines 6-29) publications. Furthermore, computer algorithms are available to further optimize selection of interfering RNA sequences (Agrawal *et al.*, page 671, second column, lines 3-8).

Structural determinants for designing more effective and stable interfering RNA molecules have also been identified (see Chiu *et al.*, *RNA*, 2003, September, 9:1034-1048; Amarzguioui *et al.*, *Nucleic Acids Res.*, 2003, Jan., 31(2):589-595, which are submitted herewith).

Recognizing that the state of the art has sufficiently developed, the Federal Circuit has held that "the complete amino acid sequence of a protein may put one in possession of the genus of DNA sequences encoding it ... one of ordinary skill in the art at the time the ... application was filed may have therefore been in possession of the entire genus of DNA sequences that can encode the disclosed partial protein sequence, even if individual species within that genus might not have been described or rendered obvious". *In re Wallach*, 71 USPQ2d 1939; 378 F.3d 1330 (CAFC 2004). The Court also cited the Patent Office's Manual of Patent Examining Procedure (MPEP), which states:

> Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. For example, in the molecular biology arts, if an applicant disclosed an amino acid sequence, it would be unnecessary to provide an explicit disclosure of nucleic acid sequences that encoded the amino acid sequence. Since the genetic code is widely known, a disclosure of an amino acid sequence would provide sufficient information such that one would accept that an applicant was in possession of the full genus of nucleic acids encoding a given amino acid sequence, but not necessarily any particular species. MPEP §2163.II.A.3.a.ii. (8th ed., rev. 2, 2001 and May, 2004).

"Moreover, we see no reason to require a patent applicant to list every possible permutation of the nucleic acid sequences that can encode a particular protein for which the amino acid sequence is disclosed, given the fact that it is, as explained above, a routine matter to convert back and forth between an amino acid sequence and the sequences of the nucleic acid molecules that can encode it." *In re Wallach*, at 1942.

While it is true that sequences and structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. For example, possession of an antibody may be demonstrated based on a description and characterization of its corresponding antigen. Disclosure of an antigen fully characterized by its structure, formula, chemical name, physical properties, or deposit in a public depository provides an adequate written description of an antibody

claimed by its binding affinity to that antigen. *Noelle v. Lederman*, 355 F.3d 1343, 1349; 69 USPQ2d 1508, 1514 (Fed. Cir. 2004) and MPEP 2163 IIA3(a). Accordingly, the teaching of the subject specification and knowledge of the sequence and structure of the s-SHIP gene provides one skilled in the art with sufficient structural and functional correlates to describe the genus of target mRNA and corresponding interfering RNA.

Thus, the applicants submit that the subject specification contains sufficient disclosure to convey to one of ordinary skill in the art that the applicants had possession of the concept of what is claimed, which is all that is necessary to satisfy the written description requirement of 35 U.S.C. §112, first paragraph. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

In view of the foregoing remarks and amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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GPL/mv
Attachments: Petition and Fee for Extension of Time
Exhibit A
Agrawal *et al.*
Amarzguioui *et al.*
Barton *et al.*
Castanotto *et al.*
Chiu *et al.*
Damen *et al.*
Donze *et al.*
Krichevsky *et al.*
McManus *et al.*
Milhavet *et al.*
Mousses *et al.*
NCBI Accession numbers AF184912 and U50040
Oliveira *et al.*
Peng *et al.*
Reich *et al.*
Rohrschneider *et al.*
Sohail *et al.*
Tuschl *et al.*
Yang *et al.*